[Cancer Biology & Therapy 5:7, 788-793, July 2006]; ©2006 Landes Bioscience

Research Paper

NF-κB Does Not Influence the Induction of Apoptosis by Ukrain

Julia Mendoza
Ruben Zamora
Juan C. Gallardo
Gisela Ceballos
Aida Aldana
Magali Espinosa
Vilma Maldonado*
Jorge Melendez-Zajgla*

Molecular Biology Laboratory; Subdireccion de Investigacion Basica; Instituto Nacional de Cancerologia; Mexico City, Mexico

*Correspondence to: Vilma Maldonado; Laboratorio de Biología Molecular; División de Investigación Básica; Instituto Nacional de Cancerología; Av. San Fernando # 22. Tlalpan 14000; México, D.F. Mexico; Tel.: 525.628.0425; Fax: 525.628.0426; Email: vilmaml@gmail.com/Jorge Meléndez-Zajgla; Laboratorio de Biología Molecular; División de Investigación Básica; Instituto Nacional de Cancerología; Av. San Fernando # 22. Tlalpan 14000; México, D.F. Mexico; Tel.: 525.628.0427; Fax: 525.628.0432; Email: jorgezajgla@gmail.com

Received 03/03/06; Accepted 03/26/06

Previously published online as a *Cancer Biology & Therapy* E-publication: http://www.landesbioscience.com/journals/cbt/abstract.php?id=2752

KEY WORDS

apoptosis, Ukrain, NF-κB, Bcl-3, IKK2, IκBα

ABBREVIATIONS

IKK2	Inhibitor of NF-κB kinase 2
NFκB	Nuclear Factor κB
Bcl-3	B-cell lymphoma 3

ACKNOWLEDGEMENTS

This project was supported by grants CONACyT-2004-C01-45728 to J.M.Z. and CONACYT-2002-C01-42039 to V.M. We thank Dr Luis Herrera for critically reading the manuscript.

ABSTRACT

Ukrain is a reaction product of different alkaloids from *Chelidonium majus L.* (celandine) conjugated with thiophosphoric acid. It has immunoregulatory effects on T lymphocyte subsets and cytotoxic and cytostatic effects on various malignant cells. Although Ukrain has been reported to induce alterations in the cell cycle and tubulin polymerization, the specific cellular target has not been described. Since antineoplasic agents induce NF-κB and their effects are regulated by this transcription factor, we investigated its possible participation in the apoptotic effects of Ukrain.

Ukrain induced apoptosis in a panel of cancer cell lines by activating the intrinsic cell death pathway, as demonstrated by the cleavage of caspase 9 and the upregulation and cleavage of caspase 3. The effect was reversible, since long exposures (24 hours or more) were needed, as verified by clonogenic assays. Gene reporter assays showed that Ukrain activated NF-κ B. Nevertheless, this activation was not required for, and did not modulate, the Ukrain effect: neither blockage of activation by a dominant negative version of Iκ-B α or a Bcl-3 siRNA, nor activation of the pathway by overexpression of IKK2, changed the response to the drug.

In conclusion, Ukrain induced apoptosis in HeLa cervical cancer cells by activating the intrinsic pathway. In contrast to other antineoplasic drugs, the effects of Ukrain were not regulated by NF-κ B.

INTRODUCTION

Ukrain (Tris(2-([5bS-(5ba,6b,12ba)]-5b,6,7,12b,13, 14-hexahydro-13-methyl[1,3] benzodioxolo[5,6-v]-1-3- dioxolo[4,5-i]phenanthridinium-6-ol]-ethaneaminyl) phosphinesulfide.6HCl) is a reaction product of different alkaloids from *Chelidonium majus L.* (celandine) conjugated with thiophosphoric acid.[1] It has immunoregulatory effects on T lymphocyte subsets [2] and is reported to exert cytotoxic and cytostatic effects on various malignant cells.[3]

The mechanism of action of Ukrain remains uncertain. Exposure of cancer cell lines to this compound results in growth inhibition concomitant with cell cycle arrest in G_2/M. This inhibition is associated with altered expression of mitotic cyclins A and B1 and of cyclin-dependent kinases CDK1 and CDK2 and the CDK inhibitor p27 is upregulated.[4] Panzer et al. found that Ukrain inhibits tubulin polymerization, leading to impaired microtubule dynamics, resulting in activation of the spindle checkpoint and thus a metaphase block.[5] In addition, Ukrain has been found to decrease the synthesis of DNA, RNA and proteins,[6] and to inhibit cellular oxygen consumption.[7] Ukrain can induce apoptosis in cancer cells in vitro[4,8,9] and in vivo[10] by a mechanism independent of changes in bcl-2 or bax expression.[9] There are two characterized signal pathways leading to apoptosis: the death receptor and the mitochondrial pathway. Both are orchestrated by the activation of caspases, a family of conserved proteases that cleave crucial cellular substrates. The mitochondrial pathway is characterized by the release of mitochondrial cytochrome c to the cytosol where, together with the adapter protein APAF-1 and procaspase 9, it assembles the apoptosome, a multiprotein complex.[11] This complex induces the activation of caspase 9, which cleaves and activates downstream caspases that cleave a broad spectrum of cellular targets, resulting in the typical apoptotic morphology. Caspases are in turn regulated by a conserved group of cellular inhibitors, the Inhibitors of Apoptosis Proteins (IAPs).[12] The IAPs must be downregulated or inhibited to allow activated caspases to cleave their substrates.[13] A key component of steady-state IAP regulation is the transcription factor NF-κ B.[14] This important regulator induces the transcription of several antiapoptotic factors including XIAP, IAP-1 and IAP-2.[15] NF-κ B is commonly upregulated in cancer

cells,[16,17] so several attempts are currently underway to block its activation in order to render cells more susceptible to antineoplasic treatments.[16,18-21] More recently, a proapoptotic role for NF-κ B has been demonstrated in various models, through the upregulation of death receptors such as CD95L, TNF and TRAIL and of transcriptional factors such as p53 and c-myc.[22,23]

Although it has been reported that Ukrain induces alterations in the cell cycle and tubulin polymerization, the specific cellular target has not been identified. Since antineoplasic agents induce NF-κ B[24] and their effects are regulated by this transcription factor,[25] we investigated the participation of NF-κ B in the apoptotic effects of Ukrain.

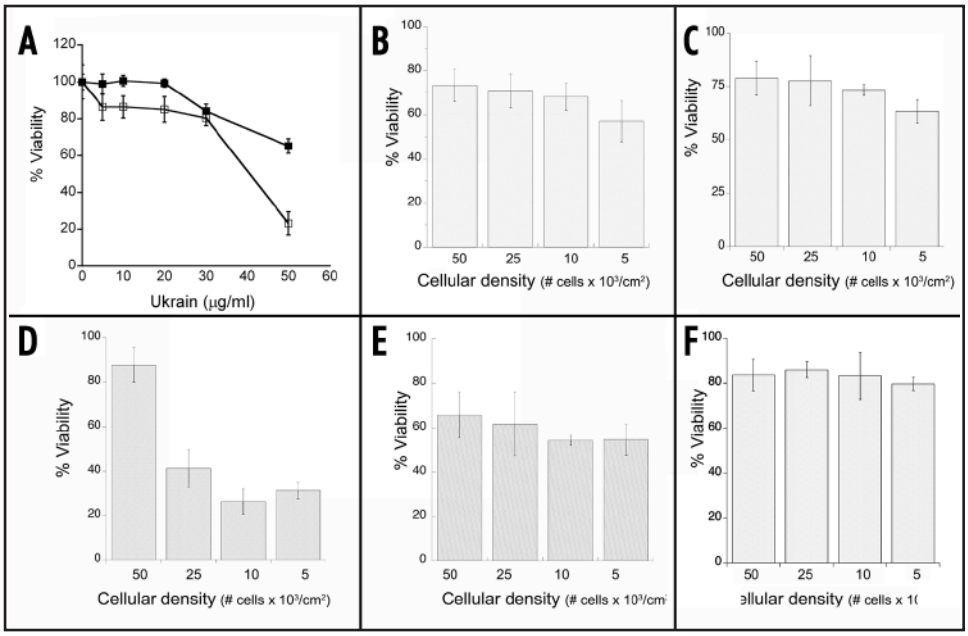

Figure 1. Effects of Ukrain on cell lines. A) Viability of HeLa cells exposed to various concentrations of Ukrain for 48 (□) or 24 (■) hours, expressed as percentage of cells exposed to vehicle (deionized water). Other panels: viability of human colon cancer SW480 cells (B), human renal carcinoma HEK293 cells (C), human cervical cancer HeLa cells (D), human osteosarcoma MG-63 cells (E) and immortalized human hTERT fibroblasts (F) exposed to 40 μg/ml Ukrain for 48 hours. The means were calculated from four independent experiments each performed by triplicate. The bars represent standard deviations.

MATERIALS AND METHODS

Cell lines and culture. Cell lines were obtained from the American Type Culture Collection (ATCC). Cells were maintained as a monolayer at 37°C and cultured in Dulbecco's modified Eagle medium containing 8% (v/v) fetal bovine serum in a humidified atmosphere with 5% (v/v) carbon dioxide in air. In order to generate a transgenic cell line defective in the canonical NF-κ pathway, we transfected HeLa cells with plasmid vectors containing a wild type or a dominant-negative Iκ-B (mutated at serine 32 and 36), kindly provided by P. Baeuerle. The S32/36 mutation prevents ubiquitination and degradation of Iκ-B protein and, thus blocks the activation of NF-κ B. Cells were then selected and analyzed (see below). Cells were thereafter termed HeKB (wild type Iκ-B) and HeKS32 (Iκ-B mutated at S32/36). To generate cells in which the pathway is constitutively activated, HeLa cells were transfected with a plasmid vector containing the IKK2 sequence; it has previously been shown that overexpression of this kinase induces constitutive activation.[26] To generate stable cell lines, cultures were selected with G418 for 4 weeks. Cells were thereafter referred as HeIKK. In order to avoid clonal variation, six clones were pooled and tested for transgene expression and NF-κ B responsiveness. In order to obtain a cell line defective in Bcl-3, we transfected HEK293 cells with a plasmid (pSIREN, Clontech) containing the sequence for producing a double stranded 23 base pair siRNA directed against Bcl-3, under the control of an U6 promoter. After selection and screening, retroviral supernatants were collected, titrated and stored in liquid nitrogen. To generate the HeBcl3- cell line, ten million HeLa cells were infected at a multiplicity of infection of 1 x 10^7, selected for 4 weeks with G418, cloned and six clones pooled. All cell culture reagents were obtained from Invitrogen (Frederick, MD).

Cellular viability and cytotoxic effects. Cells were seeded in 24 chamber dishes at the density described and exposed to several concentrations of Ukrain for 24 or 48 hours. The concentrations used to determine the IC_{50} ranged from 1 to 100 μg/ml. For the rest of the experiments, the IC_{50} value at 48 hours was used (40 μg/ml). The cells were then fixed in 70% ethanol at -20°C, washed in PBS and stained with crystal violet (1% in water). After washing, the stain was solubilized in 33% acetic acid and the absorbance was determined in an ELISA reader at 570 nm. Clonogenic assays were performed as reported previously.[27] The analyses were performed at least in triplicate in four independent experiments.

Nuclear staining. Cells were fixed with cold ethanol at 70% for 15 min and pretreated with RNAse (10 μg/ml) in PBS for 30 min at 37°C. The nuclei were stained with ethidium bromide (5 μg/ml) in PBS and washed twice with PBS. Finally, the cells were visualized with a Zeiss microscope using epifluorescence and photographed with a digital camera (Nikkon 4500).

Western blot analysis. Cells were washed with ice-cold phosphate-buffered saline and total extracts were prepared by lysis in 500 μl of Laemmli buffer, sonication for 20 seconds and centrifugation at 8000 x g for 30 minutes at 4°C. Equal amounts of protein were separated and electrophoresed on an 8–15% SDS-polyacrylamide gel (PAGE). After transferring to a PVDF membrane (Amersham-Pharmacia, UK) and blocking in nonfat milk for 1 hour, the blot was incubated with monoclonal or monospecific polyclonal antibodies against caspases 8, 9 and 3, IκB α, Bcl3 and actin (Santa Cruz Biotechnology, CA, USA). After washing, the blots were reincubated for 2 hours with a mouse or anti-rabbit IgG-HRP antibody (Amersham-Pharmacia, UK). Antibody binding was determined using enhanced chemiluminiscence (Amersham-Pharmacia, UK) with X-Omat AR films (Kodak, México).[28]

Gene reporter analyses. To measure NF-κ B activity, we established a HeLa cell line derivative (hereafter termed HeNFR) in which the plasmids pNF-kB-Luc and pBK-CMV (Stratagene CA) were cotransfected using lipofectamine 2000 (Invitrogen, MD) in a 10:1 ratio, selected for 4 weeks in G418 (Invitrogen, MD), cloned and pooled. HeNFR cells were tested for linear activation of the reporter and reproducibility using H_2O_2 and TNF-α exposure (not shown).

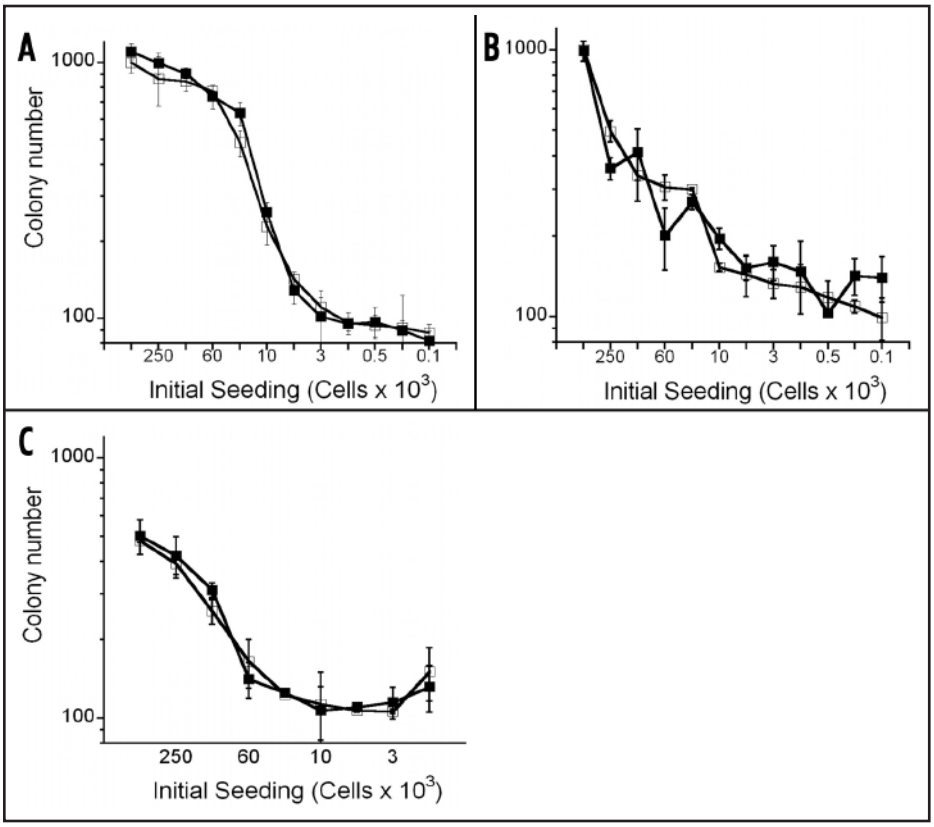

Figure 2. Effects of Ukrain on clonogenic survival of selected cancer cell lines. Cells exposed for 18 hours to the calculated IC_{50} Ukrain concentration (■) for each cell line, or to vehicle (□), were assayed for colony forming capability after 2 weeks, as described in Material and Methods. (A) HeLa cells, (B) HEK 293 cells, (C) MG-63 cells.

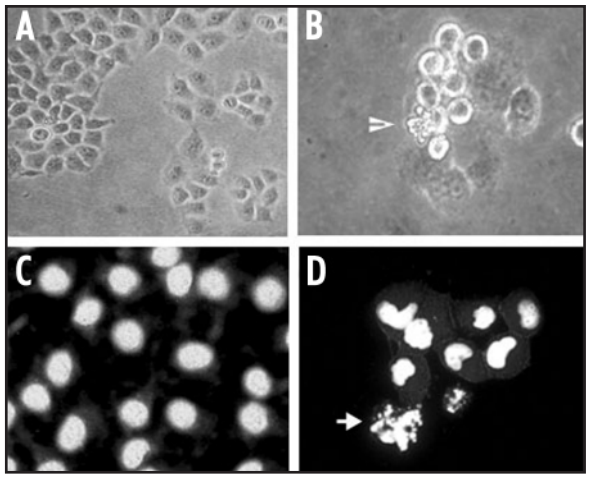

Figure 3. Morphology of HeLa cells exposed for 48 hours to a 40 μg/ml concentration of Ukrain. (A) and (B), phase contrast microscopy. (C) and (D), nuclei stained with ethidium bromide and visualized by fluorescence microscopy. Original magnification x40.

The cells were exposed to Ukrain for the times described and NF-κ B activity was analyzed by the luciferase assay system (Promega, WI), using a microplate luminometer as described by the manufacturer. All experiments were performed in triplicate in three independent experiments.

Statistical analysis. Data are expressed as means ± SD from at least three independent experiments performed by triplicate. A Student's *t* test was used to compare individual data with control value. For western blot densitometric analyses, a Bonferroni *t* test was used. A probability of $p < 0.05$ was taken as denoting a significant difference from control data.

RESULTS

To determine whether Ukrain induces apoptosis in cancer cells, we exposed HeLa cells to several concentrations (1 to 100 μg/ml) of the compound for 24 or 48 hours. As shown in Figure 1, Ukrain induced a marked dose and time-dependent decrease of viability. To examine the generality of this effect, we tested several other cancer cell lines (Fig. 1, and other data not shown). Sensitivity to Ukrain, defined as the relative decrease of cellular viability, varied among cell lines, from an IC_{50} of 43 ± 6 μg/ml in HeLa cells to 78 ± 9 μg/ml in SW480 colon cancer cells. Interestingly, nontransformed fibroblasts (hTERT cell line) were insensitive to the drug. The cytotoxic effect of Ukrain on sensitive cell lines depended on cell confluence, suggesting that it might be modulated by the cell cycle or cell-cell contact (Fig. 1). Unexpectedly, clonogenic assays using different doses of Ukrain for less than 24 hours failed to show a reproducible death effect on several cancer cell lines (Fig. 2), indicating a requirement for long time exposure to the drug. To determine whether the decrease of viability in cells exposed to Ukrain for 48 hours was due to apoptosis, we analyzed cellular and nuclear morphology using a fluorescent assay. Figure 3 illustrates the cytosolic condensation, membrane convolution and nuclear condensation and fragmentation seen in cells exposed to Ukrain, typical of apoptotic cell death. Similar results were found in all the cell lines analyzed (not shown).

During apoptosis, cell death is executed by the cleavage-dependent activation of caspases. Caspases comprise two distinct classes, the initiators and the effectors. Initiator caspases 8 and 10 mediate death factor-mediated or extrinsic apoptosis, whereas caspase 9 mediates intrinsic or mitochondria-regulated apoptosis.[12] To identify the mechanism of apoptosis induced by Ukrain, we performed western blot analyses of caspases 8 and 9 and the effector caspase 3 in HeLa cells exposed to the drug. Figure 4 shows that Ukrain induced cleavage of the caspase 9 precursor, with a concomitant increase in effector caspase 3 protein and cleavage of its proform. As expected in view of the previously-reported dependence of antineoplasic agents on the intrinsic cell death pathway,[29] caspase 8 was not cleaved. These results clearly show that Ukrain induces apoptosis in cervical cancer cells by activating the intrinsic cell death pathway.

NF-κ B is a pleiotropic transcription factor with a crucial and complex regulatory role in apoptosis. It mediates an antiapoptotic signal in response to a wide range of stimuli, including chemotherapeutic drugs.[24,25] Depending on the cellular context, this signaling can either impair the response to the death stimuli or be superseded by death signaling. Paradoxically, recent reports show that NF-κ B can also induce apoptosis in certain cells by inducing the extrinsic death pathway.[22] To assess the importance of this cascade in our model, we used a gene reporter assay to determine the rate of NF-κ B transcription in cells exposed to Ukrain. Figure 5 shows that the drug induced NF-κ B activation, as indicated by the increased enzymatic activity of the reporter protein, secreted alkaline phosphatase. To test whether NF-κ B activation modulated the cellular response to Ukrain, we created four transgenic HeLa cell lines. HeKS32 expresses a dominant negative version of Iκ B α and is thus unable to activate the canonical NF-κ B pathway; HeKB overexpresses a wild type Iκ-B α protein; HeBcl3- is a stable derivative with defective Bcl-3, a positive modulator of the noncanonical NF-κ B pathway; and finally, HeIKK cells overexpress IKK2, the main activating kinase of the canonical NF-κ B pathway.[30] When these cells were exposed to Ukrain, we found that blocking the canonical (e.g., HeKS32 and HeKB) or non canonical (e.g., HeBcl3-) NF-κ B transduction pathways did not increase the cytotoxic effects of the drug (Fig. 6), as would be expected from previous reports in which classical antineoplasic agents were used.[25] In agreement with these results, we found that the opposite approach, that is, activating the NF-κ B cascade by IKK2 overexpression, did not change the response to the drug (Fig. 6). All these results show that, even when NF-κ B is activated after Ukrain exposure, this transduction pathway neither participates nor modulates the cytotoxic response to the drug.

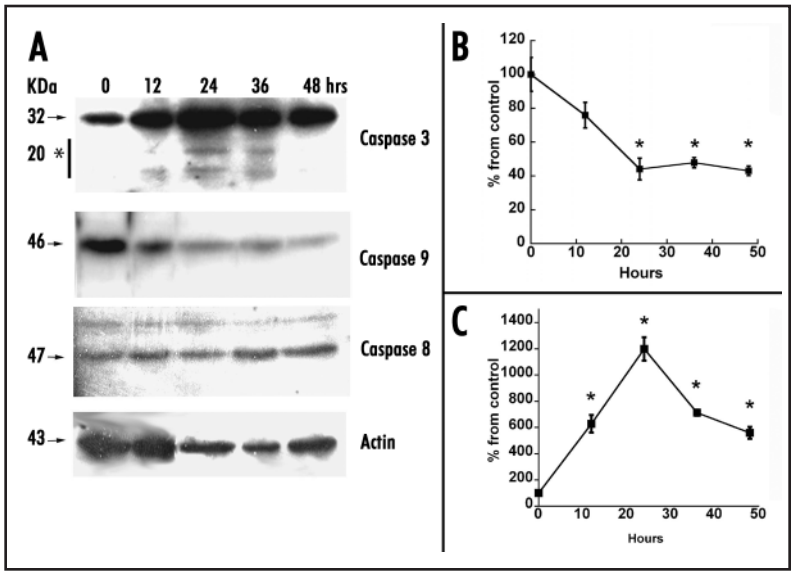

Figure 4. (A) Representative western blots of Caspases 8, 9 and 3 in HeLa cells exposed to 40 μg/ml Ukrain for the times shown. (C) Densitometric analysis of cleaved caspase 3. (B) Densitometric analysis of precursor caspase 9. These results were derived from triplicate experiments and normalized using densitometric analysis for actin. The bars represent standard deviations. *p < 0.5 by Bonferroni T-test, individual values compared to control.

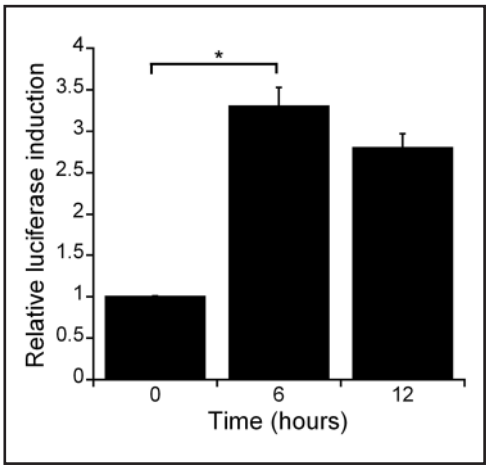

Figure 5. NF-κ B activity in cells exposed to Ukrain. HeLa cells were exposed to Ukrain and NF-κ B transactivating activity was assessed by gene reporter assays, as described in Material and Methods. Luciferase activity is presented as relative values of promoter activity. Experiments were performed by triplicate. The bars represent standard deviations. *p < 0.5 by Bonferroni T-test.

DISCUSSION

Ukrain is a novel semi-synthetic compound that has received attention because of its possible use as an effective cancer drug.[31] Nevertheless, its mechanism of action remains elusive. Initially, changes in oxygen consumption were found,[32] along with a requirement for the metabolic redox chain, but no direct cause-effect relationship was shown between these changes and the cytotoxic effects. Liepins[8] reported that Ukrain induces two modes of cell death in K562 leukemia cells, one compatible with apoptosis and the second with mitotic crisis. The induction of apoptosis by Ukrain in vitro has been further demonstrated in Chinese Hamster Ovary cells,[33] prostate cells[9] and epidermoid cancer cells, including vulvar and cervical.[4] Reduction in DNA, RNA and protein synthesis and inhibition of tubulin polymerization have been observed after exposure to Ukrain,[6,34] but no direct relationship between these cellular effects and cytotoxicity has been demonstrated. In the present paper we found that Ukrain induced apoptosis in cervical, colonic and kidney cancer cells, but not in a nontransformed cell line derived from human fibroblasts. The effect required long exposure to the compound, since the clonogenic sensitivity assays required more than 24 hours in culture to show reproducible death. This accords with the report of Panzer et al.,[35] who found that the antimitotic effects of Ukrain were reversible. Although the exact reason for this phenomenon is currently unknown, the doubling time of the HeLa cell line (22 ± 0.2 hours in our laboratory) and confluence-dependent modulation of the effect of Ukrain (Fig. 1) could indicate a cell cycle requirement for the action of the drug.

As expected in view of reports on other antineoplasic drugs,[19] apoptosis induced by Ukrain was mediated by the intrinsic pathway, as demonstrated by the cleavage of caspases 9 and 3. This is also supported by the data of Roublevskaia et al.,[36] who demonstrated that overexpression of Bcl-2, a regulator of the mitochondrial pathway, impaired responsiveness to Ukrain.

Alterations in tubulin polymerization by antineoplasic drugs usually involve the participation of the extrinsic pathway, as exemplified by paclitaxel, an anticancer compound that also targets

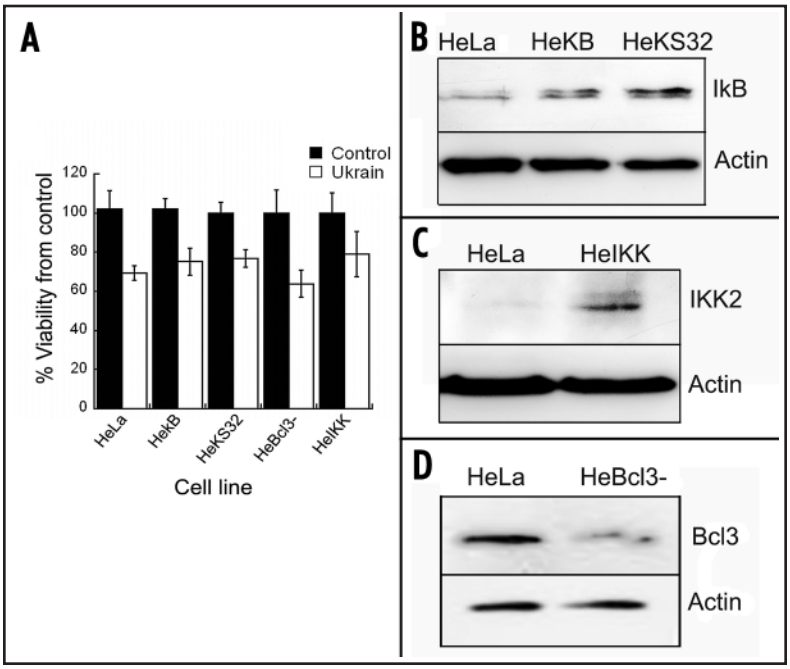

Figure 6. Effect of NF-κB inhibition on cytotoxic effects of Ukrain. (A) HeLa cell line, or stable HeLa derivative HeBcl3-, which expresses a siRNA for Bcl-3; HeKB, expressing I-κB; HeKS32, which expresses an I-κB negative dominant; and HeIKK, which overexpresses IKK2; were exposed to the IC_{50} of Ukrain for 48 hours and viability was assessed as described in Material and Methods. Experiments were performed in triplicate in three independent assays. The bars represent standard deviations. (D) Western blot assays of Bcl-3 (upper panel) and actin (lower panel) in HeLBcl3- cells to demonstrate the effect of Bcl-3 siRNA. (B) Western blot assays of IκB α (upper panel) and actin (lower panel) in HeKB cells to demonstrate expression of the transgene. (D) Western blot assays of IκB α (upper panel) and actin (lower panel) in HeKS32 cells to demonstrate expression of the transgene. (C) Western blot assays of IKK2 α (upper panel) and actin (lower panel) in HeIKK to demonstrate expression of the transgene.

microtubules. This drug induces the death receptor (DR)-mediated apoptotic pathway in HeLa cells, involving TRAIL-dependent activation of caspase-8, as well as the mitochondrial-mediated pathway.[37] Since we found no activation of caspase 8, participation of the extrinsic pathway in our model is excluded. Although further investigation is necessary, our results could indicate a secondary role for tubulin as the main target for Ukrain, as suggested by others.[34]

NF-κ B is a pleiotropic transcription factor that modulates cellular processes such as proliferation and cell death in response to external and internal stimuli such as cytokines and growth factors, inflammation and stress. It is one of the most important antiapoptotic factors, as demonstrated by the embryonic lethality induced by massive liver apoptosis in knockout mice deficient in the RelA component of NF-κ B.[38] This effect is mediated by direct upregulation of several proteins that inactivate caspases (IAPs), block mitochondrial release of apoptogenic factors (Bcl-2 family members) or displace caspase 8 from the death-inducing signalling complex (FLIP and isoforms). Recently, it has been shown that NF-κ B regulates not only antiapoptotic signals but also proapoptotic signals through the regulation of death receptors and death receptor ligands such as CD95L, TNF and TRAIL (for a review see Ref. 23) This further complexity in the regulation of apoptosis by NF-κ B probably depends on additional cross-talking cellular factors that allow for specific spatial and temporal outcomes in multicellular organisms.[39] Indeed, it has been shown that transcription factors such as Sp1[40] and TAF(II)105[41] can bind to the same promoter site as, or interact directly with, NF-κ B, regulating its specificity and activity. In view of the previously reported immunomodulatory effects of Ukrain[2,42] and the fact that NF-κ B plays a very important role in innate and adaptive immunity,[43] we reasoned that this factor could be mediating or at least regulating the response to Ukrain in cancer cells. In the present report, we show for the first time that even when Ukrain activates NF-κ B, this activation does not modify the drug response. This follows from the observation that neither blockage of canonical NF-κ B, which relies on Iκ-B degradation and the release of prototypic p50/p65 homodimers, nor the depletion of bcl-3, which is required for the transactivation activities of noncanonical p50 and p52 homo or heterodimers, inhibited the action of Ukrain. In support of these data, constitutive activation of the canonical pathway, induced by overexpression of its main activator, IKK2,[30] did not change the responsiveness to the drug. These novel results showing the activation of NF-κ B, along with recent reports demonstrating the immunoregulatory effects of Ukrain on T lymphocyte subsets and on the antitumor effect of NK cells,[2,42] provide new grounds for investigating whether NF-κ B regulation could also be responsible for the immunomodulatory effects of Ukrain.

In conclusion, we have shown that Ukrain induces apoptosis in cancer cells by activating the intrinsic death pathway by a mechanism that does not depend on the activation of NF-κ B.

References

1. Nowicky J, Greif M, Hamler F, Hiesmayr W, Staub W. Biological activity of Ukrain in vitro and in vivo. Chemioterapia 1987; 6:683-5.
2. Nowicky JW, Manolakis G, Meijer D, Vatanasapt V, Brzosko WJ. Ukrain both as an anti cancer and immunoregulatory agent. Drugs Exp Clin Res 1992; 18:51-4.
3. Uglyanitsa KN, Nefyodov LI, Doroshenko YM, Nowicky JW, Volchek IV, Brzosko WJ, Hodysh YJ. Ukrain: A novel antitumor drug. Drugs Exp Clin Res 2000; 26:341-56.
4. Roublevskaia IN, Polevoda BV, Ludlow JW, Haake AR. Induced G_2/M arrest and apoptosis in human epidermoid carcinoma cell lines by semisynthetic drug Ukrain. Anticancer Res 2000; 20:3163-7.
5. Panzer A, Joubert AM, Bianchi PC, Hamel E, Seegers JC. The effects of chelidonine on tubulin polymerisation, cell cycle progression and selected signal transmission pathways. Eur J Cell Biol 2001; 80:111-8.
6. Nowicky JW, Hiesmayr W, Nowicky W, Liepins A. Influence of Ukrain on DNA, RNA and protein synthesis in malignant cells. Drugs Exp Clin Res 1996; 22:81-91.
7. Jagiello-Wojtowicz E, Kleinrok Z, Urbanska EM. Ukrain (NSC-631570) in experimental and clinical studies: A review. Drugs Exp Clin Res 1998; 24:213-9.
8. Liepins A, Nowicky JW, Bustamante JO, Lam E. Induction of bimodal programmed cell death in malignant cells by the derivative Ukrain (NSC-631570). Drugs Exp Clin Res 1996; 22:73-9.
9. Roublevskaia IN, Haake AR, Ludlow JW, Polevoda BV. Induced apoptosis in human prostate cancer cell line LNCaP by Ukrain. Drugs Exp Clin Res 2000; 26:141-7.
10. Uglyanica KN, Maciuk JR, Fomin KA, Nefyodov LI, Kravchuk RI, Vinogradova LM, Nowicky JW, Brzosko WJ. Influence of Ukrain on patients with surgically treated breast cancer. Part IV. Electronmicroscopic and cytochemical evaluation. Drugs Exp Clin Res 1996; 22:147-53.
11. Henry-Mowatt J, Dive C, Martinou JC, James D. Role of mitochondrial membrane permeabilization in apoptosis and cancer. Oncogene 2004; 23:2850-60.

12. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. Nature Rev Mol Cell Biol 2004; 5:897-907.
13. Nachmias B, Ashhab Y, Ben-Yehuda D. The inhibitor of apoptosis protein family (IAPs): An emerging therapeutic target in cancer. Semin Cancer Biol 2004; 14:231-43.
14. Baichwal VR, Baeuerle PA. Activate NF-kappa B or die? Curr Biol 1997; 7:R94-6.
15. Van Antwerp DJ, Martin SJ, Verma IM, Green DR. Inhibition of TNF-induced apoptosis by NF-kappa B. Trends Cell Biol 1998; 8:107-11.
16. Haefner B. NF-kappa B: Arresting a major culprit in cancer. Drug Discov Today 2002; 7:653-63.
17. Rayet B, Gelinas C. Aberrant *rel/nfkb* genes and activity in human cancer. Oncogene 1999; 18:6938-47.
18. Young MR, Yang HS, Colburn NH. Promising molecular targets for cancer prevention: AP-1, NF-kappa B and Pdcd4. Trends Mol Med 2003; 9:36-41.
19. Debatin KM. Apoptosis pathways in cancer and cancer therapy. Cancer Immunol Immunother 2004; 53:153-9.
20. Nakanishi C, Toi M. Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. Nat Rev Cancer 2005; 5:297-309.
21. Monks NR, Biswas DK, Pardee AB. Blocking anti-apoptosis as a strategy for cancer chemotherapy: NF-kappaB as a target. J Cell Biochem 2004; 92:646-50.
22. Hacker H, Karin M. Is NF-kappaB2/p100 a direct activator of programmed cell death? Cancer Cell 2002; 2:431-3.
23. Aggarwal BB. Signalling pathways of the TNF superfamily: A double-edged sword. Nat Rev Immunol 2003; 3:745-56.
24. Maldonado V, Melendez-Zajgla J, Ortega A. Modulation of *NF-kappa B*, and *Bcl-2* in apoptosis induced by cisplatin in HeLa cells. Mutat Res 1997; 381:67-75.
25. Bandala E, Espinosa M, Maldonado V, Melendez-Zajgla J. Inhibitor of apoptosis-1 (IAP-1) expression and apoptosis in nonsmall-cell lung cancer cells exposed to gemcitabine. Biochem Pharmacol 2001; 62:13-9.
26. Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV. IkappaB kinase-beta: NF-kappaB activation and complex formation with IkappaB kinase-alpha and NIK. Science 1997; 278:866-89.
27. Hernandez P, Olivera P, Duenas-Gonzalez A, Perez-Pastenes MA, Zarate A, Maldonado V, Melendez-Zajgla J. Gemcitabine activity in cervical cancer cell lines. Cancer Chemother Pharmacol 2001; 48:488-92.
28. Melendez-Zajgla J, Garcia C, Maldonado V. Subcellular redistribution of HSP72 protein during cisplatin-induced apoptosis in HeLa cells. Biochem Mol Biol Int 1996; 40:253-61.
29. Hajra KM, Liu JR. Apoptosome dysfunction in human cancer. Apoptosis 2004; 9:691-704.
30. Aggarwal BB. Nuclear factor-kappaB: The enemy within. Cancer Cell 2004; 6:203-208.
31. Ernst E, Schmidt K. Ukrain—A new cancer cure? A systematic review of randomised clinical trials. BMC Cancer 2005; 5:69.
32. Philchenkov AA. Caspases as regulators of apoptosis and other cell functions. Biochemistry (Mosc) 2003; 68:365-76.
33. Kurochkin SN, Kolobkov SL, Votrin II, Voltchek IV. Induction of apoptosis in cultured Chinese hamster ovary cells by Ukrain and its synergistic action with etoposide. Drugs Exp Clin Res 2000; 26:275-8.
34. Panzer A, Hamel E, Joubert AM, Bianchi PC, Seegers JC. Ukrain(TM), a semisynthetic Chelidonium majus alkaloid derivative, acts by inhibition of tubulin polymerization in normal and malignant cell lines. Cancer Lett 2000; 160:149-57.
35. Panzer A, Joubert AM, Bianchi PC, Seegers JC. The antimitotic effects of Ukrain, a Chelidonium majus alkaloid derivative, are reversible in vitro. Cancer Lett 2000; 150:85-92.
36. Roublevskaia IN, Haake AR, Polevoda BV. Bcl-2 overexpression protects human keratinocyte cells from Ukrain-induced apoptosis but not from G_2/M arrest. Drugs Exp Clin Res 2000; 26:149-56.
37. Lee KH, Yim EK, Kim CJ, Namkoong SE, Um SJ, Park JS. Proteomic analysis of anti-cancer effects by paclitaxel treatment in cervical cancer cells. Gynecol Oncol 2005; 98:45-53.
38. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the *RelA* component of *NF-kappa B*. Nature 1995; 376:167-70.
39. Wallach D, Arumugam TU, Boldin MP, Cantarella G, Ganesh KA, Goltsev Y, Goncharov TM, Kovalenko AV, Rajput A, Varfolomeev EE, et al. How are the regulators regulated? The search for mechanisms that impose specificity on induction of cell death and NF-kappaB activation by members of the TNF/NGF receptor family. Arthritis Res 2002; 4:S189-96.
40. Hirano F, Tanaka H, Hirano Y, Hiramoto M, Handa H, Makino I, Scheidereit C. Functional interference of Sp1 and NF-kappaB through the same DNA binding site. Mol Cell Biol 1998; 18:1266-74.
41. Yamit-Hezi A, Nir S, Wolstein O, Dikstein R. Interaction of TAFII105 with selected p65/RelA dimers is associated with activation of subset of *NF-kappa B* genes. J Biol Chem 2000; 275:18180-7.
42. Grinevich Y, Shalimov S, Bendyuh G, Zahriychuk O, Hodysh Y. Effect of Ukrain on the growth and metastasizing of Lewis carcinoma in *C57BL/6* mice. Drugs Exp Clin Res 2005; 31:59-70.
43. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. Annu Rev Immunol 1994; 12:141-79.